# MISSOURI DEPARTMENT OF NATURAL RESOURCES
# AIR AND LAND PROTECTION DIVISION
# ENVIRONMENTAL SERVICES PROGRAM
# Standard Operating Procedures

SOP #: MDNR-FSS-108 EFFECTIVE DATE: November 4, 2002

SOP TITLE: Field Analysis of Fecal Coliform Bacteria

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SUMMARY OF REVISIONS: Revisions were made to section 12.3. The bacteriological analysis bench sheet used by ESP personnel was also updated.

APPLICABILITY: Applies to all field personnel who collect and analyze water samples for fecal coliforms.

DISTRIBUTION:
MoDNR Intranet
ESP FSS Supervisor
ESP FSS SOP Coordinator
ESP WQMS Supervisor

RECERTIFICATION RECORD:

| | | | | |
|---|---|---|---|---|
| Date Reviewed | | | | |
| Initials | | | | |

## 1.0 SCOPE AND APPLICABILITY

1.1 Fecal coliforms are a portion of the total group of coliform bacteria that are present in the intestines and feces of warm-blooded animals.

1.2 The presence of fecal coliform bacteria is an indication that pathogenic (disease causing) bacteria may be present. Fecal coliform analysis is used to determine the degree of pollution and/or the sanitary quality of a water body or wastewater discharge system.

1.3 The membrane filter technique involves filtering a water sample through a membrane to trap bacteria that are present in the sample. This filter is then placed on growth media and incubated at 44.5$^{o}$C for 24 hours. The combination of selective media and high temperature favors the growth of fecal coliform bacteria while suppressing the growth of non-fecal coliform bacteria.

## 2.0 PERSONNEL QUALIFICATIONS

Field personnel will have at a minimum attended the department-sponsored inspection and enforcement training or received training from a MDNR employee knowledgeable of the following standard operating procedures.

- MDNR-FSS-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times and Special Considerations*
- MDNR-FSS-002 *Field Sheet and Chain-of-Custody Record*
- MDNR-FSS-004 *Sample Numbering and Labeling*
- MDNR-FSS-005 *General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples for Streams and Wastewater Flows*
- MDNR-FSS-018 *Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab*

## 3.0 HEALTH AND SAFETY

3.1 Field personnel who are routinely exposed to wastewater (domestic or animal) are encouraged to protect themselves from water borne illnesses by wearing appropriate personal protective equipment such as clean disposable gloves and waders. They are also encouraged to frequently wash their hands with soap and water.

3.2 Personnel should participate in medical monitoring in accordance with the DEQ medical monitoring policy. All field personnel who are routinely exposed to domestic and animal waste should be familiar with the DEQ Hepatitis A Prevention vaccine policy. Both policies can be reviewed on the MoDNR's intranet home page by accessing the DEQ Health and Safety information page.

## 4.0 SAMPLING CONSIDERATIONS

As stated in *Standard Methods for the Examination of Water and Wastewater,* 1995, 19th Edition, section 9010, the examination of routine bacteriological samples cannot be regarded as providing complete information concerning water quality. The bacteriological results should always be considered in light of information available concerning the sanitary conditions surrounding the sample source. Therefore, when possible, the evaluation of water quality should be based on the examination of a series of samples collected over a known and protracted period.

## 5.0 SAMPLE COLLECTION, CONTAINERS, PRESERVATIVES AND HOLDING TIME

5.1 A grab sample containing a minimum of 100 mL of sample should be collected in a sterilized Nalgene™ bottle or sterile Whirl Pak™ bag. Refer to MDNR-FSS-005 for the proper collection of grab samples and general sampling considerations. Each sample container will be labeled with a sample number, and the date and time of collection. This information will be documented on the Chain-of-Custody Record (refer to MDNR-FSS-002 *Field Sheet and Chain-of-Custody Record* and MDNR-FSS-003 *Sample Numbering and Labeling*).

5.2 When collecting a grab sample, leave an air space within the sample container to facilitate mixing the sample by vigorous shaking before examination (refer to *Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9060*). For consistency, the USGS, National Field Manual for the Collection of Water-Quality Data, Book 9, Chapter A7, Biological Indicators recommends shaking the sample 25 times before examination. This will ensure an even distribution of fecal coliform bacteria and break up any particles in the sample.

5.3 When collecting grab samples using a Nalgene™ bottle, grab samples should be collected by holding the sample bottle near its base and plunging it (neck down) below the surface of the water. The bottle should be turned until the neck of the bottle points slightly upwards. If a water current is present, the mouth of the bottle should be directed into the current. If possible, the sample bottle should be capped while the bottle is still submerged below the surface of the water.

5.4 When collecting grab samples using a Whirl Pak™ bag a Whirl Pak™ water scoop should be used. The security tab should be removed, the tabs pulled out, and the wire ends secured to the water scoop prior to submerging the Whirl Pak™ bag. Submerge the bag under the water surface and slowly move the bag into the current (if present). To aid in filling the Whirl Pak™ bag the bottom of the bag may be held as the bag is moved into the current. The bag should be filled to the 100 mL fill line. If possible, the bag should be closed while it is submerged below the surface of the water. Remove the water scoop, grip the wire ends and secure the bag closed by carefully whirling the bag in at least three complete revolutions. Then turn the wire ends inward to the opposite face of the fold and twist together.

5.5 For chlorinated discharge streams, samples shall be dechlorinated with 0.25 mL of a 10% solution of sodium thiosulfate or a single tablet of sodium thiosulfate for each 250 mL of sample. Sodium thiosulfate can be pre-added or added to the sample container immediately following collection. This will neutralize 15 mg/L of residual chlorine. If the sodium thiosulfate is pre-added, it is important not to rinse the sample bottle before collecting the final sample.

5.6 For unchlorinated discharge streams, samples should be collected directly into the sterilized sample container. The addition or presence of sodium thiosulfate in an unchlorinated will not compromise the sample.

5.7 All samples will be kept on ice and analysis will begin within 6 hours from the time of collection. To prevent the Whirl Pak™ bags from becoming smashed and/or leaking their contents, the bags shall be placed within a container (e.g. beaker) then stored on ice.

## 6.0 GLASSWARE AND EQUIPMENT

- 250 mL sterilized Nalgene™ bottles or sterile Whirl Paks™ bags
- Sodium thiosulfate tablets or 10% sodium thiosulfate solution (dechlorinating agent)
- Whirl Pak™ water scoop
- Sterile, bacteriological 10-11 mL pipettes
- Vacuum pump and backup Nalgene™ hand pump with appropriate tubing
- Milk bottles containing sterilized buffered rinse water
- 1 Liter vacuum filter flask
- 99 mL dilution bottles containing sterile, buffered dilution water
- Alcohol lamp or candle
- Forceps: sterilized by dipping in ethanol and flaming
- Ethanol for sterilizing forceps
- 50 mL glass beaker to contain ethanol during the procedure
- Sterile petri dishes
- Absorbent pads*
- Sterile membrane filters, white, 0.45 µm pore size, 47 mm diameter with grid face*
- Sterile membrane filtration apparatus (consisting of filter base and stainless steel or Nalgene™ funnel)
- Solid block incubator
- 10x magnification source and hand tally
- Bacteriological analysis bench sheet (Appendix A)
- Permanent marker or grease pencil
- m-FC broth with or without rosalic acid
- 1 mL pipette and pipette tips

* The absorbent pads and sterile membrane filters shall be marked with the date received. The absorbent pad container shall also be marked with the date that it was opened.

## 7.0 REAGENTS

- m-FC broth with rosalic acid: Rosalic acid is used to inhibit the growth of non-coliform bacteria. m-FC broth with rosalic acid should be used when analyzing samples collected from systems that are high in turbidity or non-coliform bacteria, such as wastewater treatment facilities without disinfection, stormwater runoff from animal feedlots or other similar types of waste streams.

- m-FC broth without rosalic acid: Should be used when analyzing samples collected from systems that are considered to have low non-coliform bacteria and turbidity concentrations, such as, streams, groundwater, disinfected (chlorinated, ultraviolet, ozone) wastewater discharges or domestic water supplies.

### 7.1 m-FC broth preparation:

7.1.1 The media can be prepared by either Chemical Analyses Section (CAS) or Water Quality Monitoring Section (WQMS) personnel, depending on arrangements made by the field investigator. In any case, the media should be made no more than 48 hours in advance of the sample collection.

7.1.2 Dissolve 3.7 grams m-FC broth base in 100 mL deionized water. Stir frequently while heating to near boiling. Remove from heat and allow to cool for several minutes prior to use. The final pH should be 7.4 ± 0.2. (refer to *Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9222 D*). According to Standard Methods, the pH of a small aliquot of the broth can be checked with pH paper. The final pH can be adjusted with 1 N HCl. If rosalic acid is to be used, add 1 mL of 1% rosalic acid to every 100 mLs of broth.

1% Rosalic acid preparation:

- A 1% solution can be made by dissolving 0.1 gram of rosalic acid crystals in 10.0 mL of 0.2N sodium hydroxide (NaOH).

- Excess rosalic acid solution can be stored for up to one week in a refrigerator, but if it turns brown from its normal reddish color when fresh, it should be discarded.

0.2 N NaOH preparation:

- Dissolve 4 grams NaOH in deionized water and dilute to 500 mL.

7.1.3 To prevent moisture loss, the broth should be stored in a tightly sealed sterile container or poured directly into sterile petri dishes. The broth should be kept cool or refrigerated at 4 - 8 °C prior to use. Any unused broth should be discarded after 96 hours (refer to *Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9222 D*).

7.2 m-FC Broth Ampules

7.2.1 Prepared individual sterile m-FC broth ampules may be purchased from an outside company that specializes in microbiological equipment. However, for QC purposes each lot number received shall be checked against a standard laboratory preparation of m-FC broth, described above, prior to use, then periodically as it approaches its expiration date (refer to *Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, sections 9020 B and 9222 D*).

7.2.2 Each case of broth ampules purchased shall be checked for the expiration date and marked with the date received. The ampules shall be kept cool or refrigerated at 4 - 8 °C prior to use. All purchased broth will be used on a first-in, first-out basis. Broth that is caked, discolored, or shows other deterioration shall be discarded (refer to *Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9020 B*).

7.3 Buffered dilution water preparation:

Stock phosphate solution:

- Dissolve 34.0 g potassium dihydrogen phosphate ($KH_2PO_4$) in 500 mL of reagent-grade water, adjust to pH 7.2 ± 0.5 with 1 N NaOH, and dilute to 1 L with reagent-grade water.

- Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride solution (81.1 g $MgCl_2 \bullet 6\ H_2O$/L reagent-grade water) to 1 L reagent-grade water. Dispense in amounts that will provide 99 ± 2.0 mL after autoclaving for 15 minutes (refer to *Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9050 C* ).

8.0 PREPARATION AND SET-UP

8.1 Field personnel should complete the necessary information on the bacteriological analysis bench sheet (refer to Appendix A) prior to analysis:

- Parameter being monitored
- Property number of the block incubator used
- Incubation temperature
- Dates and times of sample collection and the initials of the sample collector
- Absorbent pad and membrane filter lot numbers, and brand names
- Reagent lot numbers and brand names, and/or the dates reagents were prepared and the preparer's initials
- Indicate whether the pH of the broth was within acceptable range (The final pH should be 7.4 ± 0.2)
- Sample numbers
- Dilution factors and multiplier

8.2 It shall be indicated on the bacteriological analysis bench sheet whether m-FC broth with or without rosalic acid was used during the analysis (refer to Appendix A).

- If the m-FC broth is prepared at ESP: The analyst shall record the m-FC broth base and rosalic acid reagent lot numbers and date prepared in the appropriate location.

- If the m-FC broth ampules are used: The analyst shall record the manufacturer's lot number, and brand name.

8.3 Along with the sample collector's initials, the date and time the sample was collected shall be recorded on the bacteriological analysis bench sheet. If more than one sample is collected, record the time that the first sample was collected. This will document that the analysis was begun within the 6 hour holding time.

8.4 Several dilutions should be analyzed to bracket the desired fecal colony counts (between 20 – 60 blue colonies/plate. When the bacterial density of the sample is unknown, filter several volumes and/or dilutions to achieve a countable density. Estimate the volume and/or dilution that is expected to yield the desired colony count. Refer to Table I. for assistance in determining the appropriate filter volumes (*Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9222 D.*)

Table I. Suggested Sample Volumes for Membrane Filter Fecal Coliform Test

| Water Source | Suggested Volume to be Filtered | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **100** | **50** | **10** | **3** | **1** | **0.1** | **0.01** | **0.001** |
| Lakes, reservoirs | X | X | | | | | | |
| Wells, springs | X | X | | | | | | |
| Water supply intake | | X | X | X | X | | | |
| Natural bathing waters | | X | X | X | X | | | |
| Sewage treatment plant | | | X | X | X | X | | |
| Farm ponds, rivers | | | | X | X | X | X | |
| Stormwater runoff | | | | X | X | X | X | |
| Raw municipal sewage | | | | | | X | X | X |
| Feedlot runoff | | | | | | X | X | X |

*Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9222 D*

8.5 All dilutions shall be predetermined and prepared prior to filtering. Bacteria should not be suspended in the dilution water for more than 30 minutes at room temperature because death or multiplication may occur.

8.6 Prepare dilutions in the dilution bottles as outlined in sections 8.6.1- 8.6.3 and Appendix B. To avoid confusion, field personnel should write the corresponding sample number on the dilution bottle and corresponding cap.

8.6.1 Dilution #1: Vigorously shake the raw sample at least 25 times. With a clean and sterile pipette, withdraw 1 mL of raw sample. Add the 1 mL of raw sample to the first 99 mL dilution bottle without touching the sides of the dilution bottle or the dilution water. Place the pipette back into the raw sample container. Number the dilution bottle and cap with the number 1. Cap the container and shake the bottle vigorously at least 25 times.

8.6.2 Dilution #2: With a clean and sterile pipette, withdraw 1 mL of dilution #1. Add the 1 mL of dilution #1 to a second 99 mL dilution bottle making certain not to touch the sides of the bottle or the dilution water. Place the pipette back into the dilution #1 bottle. Number the second dilution bottle and cap with the number 2. Cap the container and shake the bottle vigorously at least 25 times.

8.6.3 Dilution #3: With a clean and sterile pipette, withdraw 1 mL of dilution #2. Add the 1 mL of dilution #2 to a third 99 mL dilution bottle making certain not to touch the sides of the bottle or the dilution water. Place the pipette back into the dilution #2 bottle. Number the third dilution bottle and cap with the number 3. Cap the container and shake vigorously at least 25 times. Place a clean and sterile pipette in the dilution #3 bottle.

8.7 Petri dishes shall also be marked with the sample number and the corresponding dilution factor using a permanent marker or wax pencil.

## 9.0 ANALYTICAL PROCEDURES

9.1 The incubator should be plugged in and allowed to warm to the desired temperature for at least one hour prior to conducting the analysis.

9.2 Using aseptic techniques, sterilized forceps or the absorbent pad dispenser should be used to place 1 absorbent pad in each petri dish. Using a sterilized pipette, pipette approximately 2 mL or pour one ampule of m-FC broth into each dish. Once the absorbent pad is completely saturated, pour off and discard the excess broth.

9.3 Using aseptic techniques, cooled and sterilized forceps should be used to place a membrane filter, grid side up, on the filtration apparatus. Close the apparatus tightly to prevent leakage from occurring during the filtration process.

9.4 To avoid cross-contamination, filtration should begin with the most diluted volume and end with the most concentrated volume per sample number.

9.5 To ensure an even distribution of fecal coliform bacterial and to break up particles, the sample shall be thoroughly mixed by shaking at least 25 times just prior to withdrawing each sample volume to be filtered.

9.6 Without touching the sides of the filter funnel, pipette the appropriate amount of sample into the filtering apparatus. Filter the sample just enough to draw the sample volume completely through the filter apparatus and into the filter flask. To prevent damaging and/or stressing the fecal coliform bacteria, stop filtering and release pressure from the vacuum pump or Nalgene™ hand pump immediately after the sample volume has been pulled through the filter apparatus.

9.7 Rinse the filter apparatus by filtering 3 aliquots (10-15 mLs each) of sterilized buffered rinse water and filter again. The rinse water shall be poured around the inside perimeter of the filter flask to wash down any remnants of the sample and prevent sample carryover to the next plate from occurring.

9.8 To evenly disperse the sample across the membrane filter when filtering small aliquots of sample (between 1-10 mLs), approximately 10 mLs of rinse water should be added to the filtering apparatus prior to filtering the sample volume.

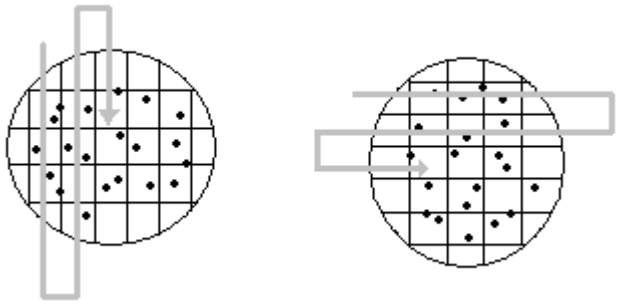
9.9 Carefully remove the filter funnel from the filtering apparatus with one hand. To prevent contamination of the filter funnel, it should never be placed on the counter top or any other potentially contaminated surface. With the opposite hand and using cooled and sterilized forceps, grasp the filter membrane along the outside edge and gently remove the membrane filter. Return the filter funnel to the filter apparatus. Carefully place the membrane filter in the corresponding prepared petri dish, grid side up, and using a rolling action to avoid trapping air bubbles between the membrane and the underlying pad.

9.10 To prevent moisture loss and to ensure the bacteria have a constant supply of nutrients, the petri dishes should be tightly closed and placed inverted in the preheated block incubator. The petri dishes should be randomly distributed throughout the incubator. Note the date, start time of incubation and the analyst's initials on the bacteriological analysis bench sheet. Incubate petri dishes at 44.5°C for 24 (± 2) hours.

9.11 When 24 (± 2) hours has elapsed, remove the petri dishes from the incubator, record the date, time, and the analyst's initials on the bacteriological analysis bench sheet.

9.12 Colonies produced by fecal coliform bacteria on m-FC broth media are various shades of blue while non-fecal coliform colonies are gray to cream colored (refer to *Standard Methods for the Examination of Water and Wastewater, 1995, 19th Edition, section 9222 D.*). Using 10x magnification and the hand tally, count all blue colonies present on each plate, and record on the bacteriological analysis bench sheet. All plates should be counted immediately following incubation.

Refer to the following diagram for a method of counting colonies on membrane filters with grids.



9.13 Refer to Appendix D for possible procedural errors that may invalidate a plate count. The validity of the plate counts is subjective and is dependent upon the number of colonies present. However, if a portion of the sample volume is lost due to leakage from the filter funnel the sample should be considered invalid and the plate count discarded.

## 10.0 QUALITY CONTROL (QC)

10.1 Field duplicates should be collected and analyzed at a minimum frequency of 10 percent (1 in every 10 samples). For every filtration series a lab duplicate should be conducted and recorded as "duplicate" on the bacteriological analysis bench sheet.

10.2 Negative controls are run at the beginning and end of each set of samples. These consist of sterilized rinse water (30-50 mLs) filtered and incubated in the same manner as the samples being analyzed. The lack of bacterial growth on negative controls indicates that the procedures, reagents and equipment used are acceptable and the sample results are valid. If numerous samples are being run, additional negative controls (or filter blanks) should be run between sample numbers to ensure good filtering and rinsing techniques are conducted. Any additional negative controls shall be documented on the bacteriological analysis bench sheet.

10.3 If there is bacterial growth on any of the negative controls, the sample results may be invalid and may need to be discarded or interpreted with caution. One or more colonies on a negative control may indicate inadequate sterilization of either the equipment or the buffered water or inadequate rinsing during the analytical technique (refer to *USGS, National Field Manual for the Collection of Water-Quality Data, Book 9, Chapter A7, Biological Indicators*).

10.4 A positive control consisting of a sample known to contain fecal coliform bacteria (e.g. influent, unchlorinated effluent, etc.) should also be run with each set of

samples. Growth of fecal coliforms on a positive control indicates that the media and other test conditions are acceptable for the growth of fecal coliforms and the sample results are therefore valid.

10.5 If there is no bacterial growth on the positive control, the analyst's supervisor should be notified and the results interpreted with caution.

10.6 For consistency and to ensure that accurate fecal coliform counts are conducted, each plate should be counted at least two times (by counting once, turning the plate a quarter turn and counting a second time) or the counts should be verified by another person. If counts differ more than 5% of the initial count then the plates shall be recounted and re-verified. The verification method and counts shall be documented on the bacteriological analysis bench sheet.

## 11.0 INTRALABORATORY QUALITY CONTROL

11.1 As stated in the *Standard Methods for the Examination of Water and Wastewater,* 1995, 19th Edition, section 9020 B, special problems exist in microbiology because analytical standards, known additions, and reference samples are usually not available. To document and ensure the validity of the tests, QC procedures are conducted to control and/or to improve the following procedures:

- sampling techniques
- sample storage and holding
- personnel
- equipment
- supplies
- media
- analytical testing

11.2 The QC checks should be conducted once a month or for every sampling event by the analyst using a known fecal coliform source (e.g. wastewater treatment plant influent). The QC check will be conducted by filtering a series of 10 aliquots of a sample of a 0.001 dilution (which has been documented to obtain counts between 20 - 60 colonies/100 mL). The QC bacteriological analysis bench sheet will be maintained by the WQMS for future reference.

## 12.0 CALCULATIONS

12.1 When reporting results, choose the plate with the most acceptable results (between 20 - 60 colonies/plate) and calculate as follows:

**# Coliform colonies/100 mL = (colonies per plate) x (multiplier)**

12.2 Use the Fecal Coliform Calculations in Appendix C for the qualifying statement that is most appropriate for each sample number. See the example in Table II.

Table II. Example of Fecal Coliform Calculations and Qualifying Statements

| Sample Number | Dilution | Multiplier | Colonies per plate | Colonies per 100mL | Selected value col./100mL | Qualifier |
|---|---|---|---|---|---|---|
| 0007865 | 0.001 | 100,000 | 16 | 1,600,000 | 430,000 | A |
| | 0.01 | 10,000 | 43 | 430,000 | | |
| | 0.1 | 1,000 | 69 | 69,000 | | |
| | 1.0 | 100 | 120 | 12,000 | | |
| | 10.0 | 10 | TNTC | TNTC | | |
| 0007866 | 0.01 | 10,000 | TNTC | TNTC | 600,000 | E |
| | 0.1 | 1,000 | TNTC | TNTC | | |
| | 1.0 | 100 | TNTC | TNTC | | |
| | 10.0 | 10 | TNTC | TNTC | | |
| | 20.0 | 5 | TNTC | TNTC | | |
| 0007867 | 0.01 | 10,000 | 0 | 0 | <5 | C |
| | 0.1 | 1,000 | 0 | 0 | | |
| | 1.0 | 100 | 0 | 0 | | |
| | 10.0 | 10 | 0 | 0 | | |
| | 20.0 | 5 | 0 | 0 | | |

Refer to Appendix D for additional examples of fecal coliform calculations, plate counts and possible errors.

12.3 Record the results and the appropriate qualifier on the bacteriological analysis bench sheet. The analyst will then enter the results into the Laboratory Information Management System (LIMS). The data is then verified by the unit supervisor and approved by the Section supervisor. The completed bench sheet will be maintained by the WQMS.

12.4 All plates shall be properly disinfected and discarded. Plates shall be discarded immediately after all results have been reported. Plates shall be autoclaved at 121º C for a minimum of 30 minutes before discarding (refer to *USGS, National Field Manual for the Collection of Water-Quality Data, Book 9, Chapter A7, Biological Indicators*). If a small number of plates are to be discarded, the plates may be disinfected by chlorinating. Personnel shall open the plates and place in 50 % chlorine solution and allowed to disinfect for several minutes. The chlorine solution can then be further diluted and poured off, and the plates discarded.

## 13.0 REFERENCES

MDNR-FSS-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Considerations*

MDNR-FSS-002 *Field Sheet and Chain-of-Custody Record*

MDNR-FSS-003 *Sample Numbering and Labeling*

MDNR-FSS-005 *General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows*

MDNR-FSS-018 *Sample Handling: Field Handling, Transportation, & Delivery to the ESP Lab*

*Standard Methods for the Examination of Water and Wastewater*, 1995, 19th Edition.

*USGS, National Field Manual for the Collection of Water-Quality Data*, Book 9, Chapter A7, Biological Indicators, 1997.

# APPENDIX A
## Bacteriological Analysis Bench Sheet

# DEPARTMENT OF NATURAL RESOURCES
# FECAL COLIFORM ANALYSIS
# BENCH SHEET

| | BRAND | LOT NUMBER | EXPIRATION DATE |
|---|---|---|---|
| **ABSORBENT PADS** | | | |
| **FILTER PADS** | | | |
| **RINSE WATER** | | | |
| **DILUTION WATER** | | | |
| **m-FC BROTH BASE** | | | |
| **ROSALIC ACID** | | | |

**m-FC BROTH PREPARATION:**

PREPARED BY:_____ DATE:_____

Was pH of broth within acceptable range: Yes or No.
If No, what pH adjustments were made?_____

_____

**SOURCE OF POSITIVE CONTROL**:_____

DATE/TIME COLLECTED:_____

**CONTROL COUNTS:**

POSITIVE CONTROL:_____colonies/plate, NEGATIVE CONTROL #1: _____colonies/plate

NEGATIVE CONTROL #2:_____colonies/plate, NEGATIVE CONTROL #3:_____colonies/plate

OTHER CONTROLS:_____

**VERIFICATION METHOD (check one):**

☐ PLATES COUNTED TWICE BY ANALYST

☐ PLATES COUNTS VERIFIED BY:_____

**COMMENTS**:_____

_____

_____

Data Verified by:_____ Date:_____

Data Approved by:_____ Date:_____

# DEPARTMENT OF NATURAL RESOURCES
# FECAL COLIFORM ANALYSIS
# BENCH SHEET

INCUBATOR USED_____ PROPERTY NUMBER_____

INCUBATION TEMPERATURE: AT START_____ °C, AT COMPLETION_____ °C

| | DATE | TIME | ANALYST |
|---|---|---|---|
| **SAMPLE COLLECTED** | | | |
| **INCUBATION STARTED** | | | |
| **INCUBATION COMPLETED** | | | |

| SAMPLE NUMBER | DILUTION | MULTIPLIER | COLONIES PER PLATE | COLONIES PER 100 ML | SELECTED VALUE COL./100 ML | QUALIFIER |
|---|---|---|---|---|---|---|
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| SAMPLE NUMBER | DILUTION | MULTIPLIER | COLONIES PER PLATE | COLONIES PER 100 ML | SELECTED VALUE COL./100 ML | QUALIFIER |
|---|---|---|---|---|---|---|
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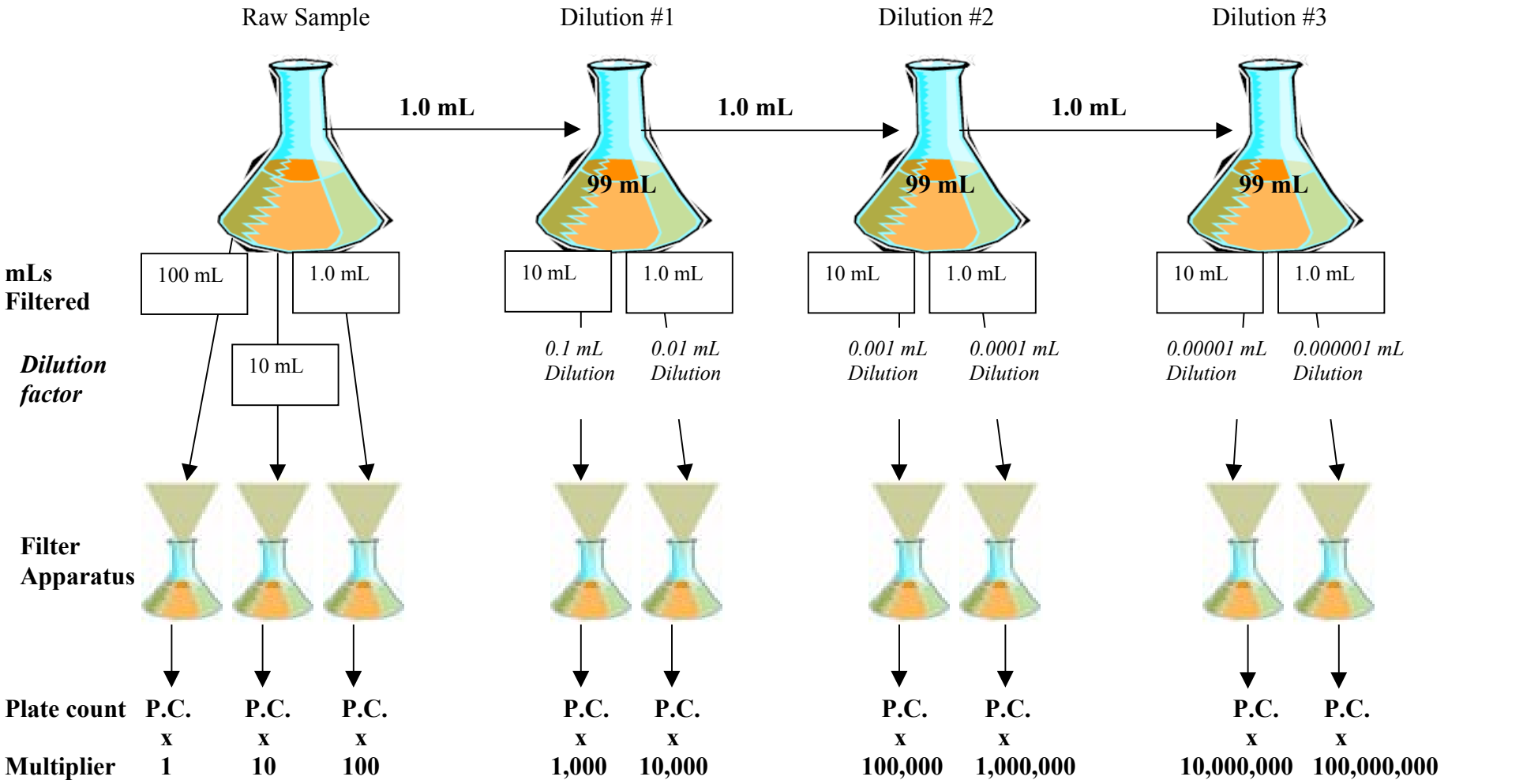
| QUALIFYING STATEMENTS: | |
|---|---|
| **TNTC** | Too Numerous To Count |
| **A** | Acceptable result, 20-60 Blue colonies per plate. |
| **B** | Estimated Count, <20 Blue colonies per plate |

| QUALIFYING STATEMENTS (CONT.): | |
|---|---|
| **C** | "<" value, No colonies, value based on largest single volume filtered. |
| **D** | Estimated count, >60 countable Blue colonies per plate |
| **E** | ">" value, colonies TNTC |

# APPENDIX B
## Bacteria Sample Dilution Procedure
## (Membrane Filter Method)

**Appendix B**
**Bacteriological Sample Dilution Procedure**
**(Membrane Filter Method)**




**The plate count multiplied by the multiplier equals the number of colonies per 100 mLs**

# APPENDIX C
# Fecal Coliform Calculations

# APPENDIX C
# MDNR-FSS-108
# Fecal Coliform Calculations

The most reliable results are obtained if the number of colonies counted is between 20 and 60. Counts of less than 20 (<20) and greater than 60 (>60) may also be used but are considered less reliable. Two hundred (200) is the maximum number of colonies that are considered countable. Results above this are reported as Too Numerous to Count (TNTC). In order to produce a countable plate (1-200), a wide range of dilutions should be prepared instead of duplicates of the same dilution. This will not always result in a count of 20-60.

When multiple dilutions are prepared, the results will not be the same, so the analyst must choose and report the best number available. Use the following qualifying statements and guidelines in your selections:

Qualifying Statements –

A. Acceptable Results -- 20-60 blue colonies per plate.

B. Estimated Count -- <20 blue colonies per plate.

C. "<" Value -- no colonies -- value based on largest single volume filtered.

D. Estimated Count -- > 60 countable blue colonies per plate.

E. ">" Value -- colonies too numerous to count.

IF, one or more are between 20-60:

THEN calculate Col/100 ml for each of these plates and determine the arithmetic average of these numbers. Disregard counts not between 20 and 60.

REPORT the arithmetic mean with qualifier A.

IF, all counts are less than 20:

THEN use the highest count observed, a single plate count. Disregard the other counts, do not calculate an average.

REPORT the actual number calculated with qualifier B.

## Appendix C (Continued)

IF, there are no colonies, zero counts:

THEN assume one (1) count was observed on the LARGEST volume filtered and calculate colonies/100 ml.

REPORT less than (<) the calculated number with qualifier C.

IF, all counts are greater than 60 but still countable, less than 200 (disregard TNTC)

THEN use the counts from the SMALLEST volume filtered and disregard the others.

REPORT the number calculated with qualifier D.

IF, all plates are TNTC:

THEN assume 60 counts were observed on the SMALLEST volume filtered and calculated colonies/100mL.

REPORT greater than (>) the number calculated with qualifier E.

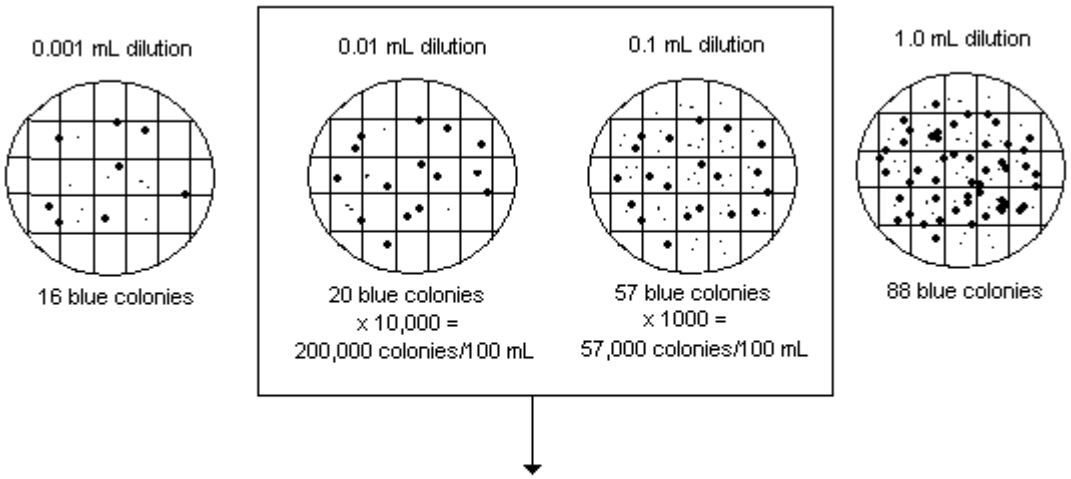
IF, there are counts less than 20 and greater than 60:

THEN use count from the LARGEST volume filtered to calculate colonies/100mL.

REPORT the number with qualifier B or D.

# APPENDIX D
## Fecal Coliform Calculations, Plate Counts and Possible Errors

# Appendix D
## Examples of Fecal Coliform Calculations, Plate Counts and Possible Errors




These plate counts are both within 20-60 blue colonies per plate. Therefore, for this sample the arithmetic average was calulated and all other counts not between 20-60 discarded.

The arithmetic mean (128,500 colonies/100 mL) was reported with qualifier A.

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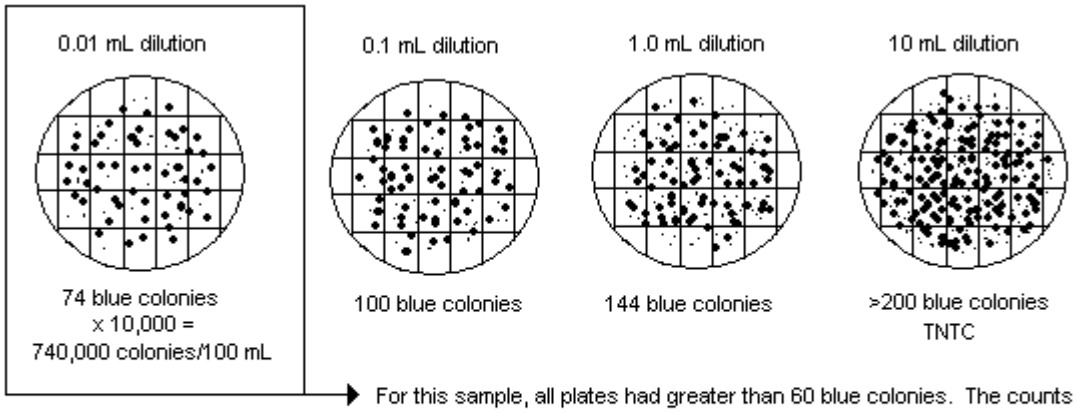


For this sample, all plates had greater than 60 blue colonies. The counts from the smallest volume filtered was used and all other plate counts were discarded.

The calculated value was reported with qualifier D.

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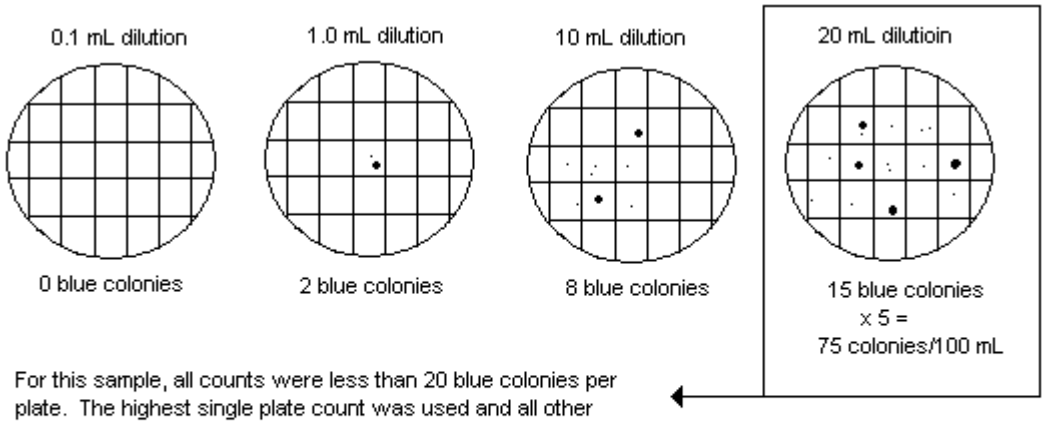
## Appendix D (cont.)
## Examples of Fecal Coliform Calculations, Plate Counts and Possible Errors




For this sample, all counts were less than 20 blue colonies per plate. The highest single plate count was used and all other counts were discarded.

The calculated value was reported with qualifier B

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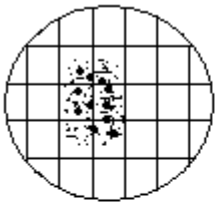
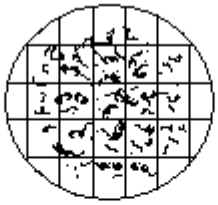
## Possible Errors



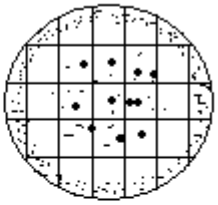
Colonies are concentrated in one area.

Sample was not evenly dispersed across the membrane prior to filtration.



Colonies look smeared.

Excess broth was not poured from plates or sample was not completely filtered through the apparatus.



Several colonies are located around the perimeter of the plate.

The filter funnel was not tightly sealed and sample leaked from the filter base.